Recent Findings with Computerized Methods for Scalp Hair Growth Measurements

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Sensitive tools have been developed in order to monitor hair loss and treatment responses. Recently the TrichoScan was presented (by RH) as such a method which combines epiluminescence microscopy (ELM) with automatic digital image analysis. Herewith new TrichoScan data obtained from 10 women and 21 men with androgenetic hair loss after 6 mo of treatment with 5%-minoxidil are presented. Even in this small cohort of patients, we noticed a significant increase of hair density, cumulative hair thickness and terminal hair counts. Alternative methods were developed during a human alopecia investigation and research technology (HAIR Technology®) programme at Skinterface. This involves contrast-enhancement, image acquisition, and processing by qualified technicians followed by computer-assisted image analysis. The specific identification of exogen hair, further adds to this very refined non-invasive investigative method for hair follicle function investigation. Regional variations of hair growth dynamics do exist in the human scalp such as in female patients complaining of hair loss, scalp hair density and growth on top of the head differs significantly from the occipital site. Finally, from transversal studies and from detailed monitoring of subsequent hair cycles during longitudinal studies, data were obtained that support the fact that shortening of hair cycle, slowing down of growth rates and thinning of hair shafts are heralding hair miniaturisation. In the workshop the TrichoScan, the method of Canfield and Skinterface have been shown.

Key words: computer-assisted hair analysis/hair/male and female alopecia/treatment/TrichoScan
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Recently, the TrichoScan has been described (Hoffmann, 2001, 2003) as a method which combines standard epiluminescence microscopy (ELM) with automatic digital image analysis for the measurement of all important hair parameters *in situ*. Herewith the application of the technique is demonstrated with new data from 10 women and 21 men with androgenetic hair loss after 6 months of treatment with 5% minoxidil. For this purpose a total of 31 persons (10 females, age range 25–48 y, mean 34 y; 21 males, age range 26–39 y, mean 32 y) underwent the study. All patients had recognized progressive thinning of hairs and hair loss for more than 2 y. All presented the clinical finding of mild to moderate androgenetic alopecia (AGA) with various degrees of involvement. Subjects with other forms of alopecia were excluded from the study. All patients were treated unblind with Regaine (5% minoxidil) for 6 mo. All patients actively treated had had no treatment whatsoever for hair loss at least 1 mo before initiating this trial. The measured parameters were hair density, cumulative hair thickness, vellus-like (<40 μm thick) and terminal hair ($\geqslant 40$ μm thick) count at the vertex in 10 males and 10 females and at frontal balding in another 9 males.

A transitional area of hair loss between normal hair and the balding area was defined and an area of 1.8 cm^2 was clipped. Some clipped areas were landmarked with a central, single red tattoo. The clipped hairs within the target area were dyed for 12 min with a commercially available solution and thereafter cleaned with an alcoholic solution and digital images were obtained at 40-fold (analyzed area: 0.225 cm^2) magnification by means of a digital ELM system (Fotofinder DERMA, Teachscreen Software, Bad Birnbach, Germany), whereas the area was still wet. This digital camera is equipped with a rigid contact lens, which ensures that the images are always taken at the same distance from the scalp. Due the fact that the camera must be pressed onto the scalp, the hairs are always flattened. Images were taken at day 0 immediately after clipping and six months after the initial visit, respectively.

For the variables hair density, cumulative thickness, vellus-like and terminal hair count the differences between the results after 6 mo and the baseline were calculated. Using this approach we noticed a significant and meaningful increase of hair density (Fig 1), cumulative hair thickness and terminal hair counts (Table I). Human scalp hairs do not grow singly, but rather in groups. These so-called follicular units contain several hairs and their number is not constant during life. During androgenetic alopecia individual hairs within follicular units miniaturize and eventually cannot be seen macroscopically. As a consequence follicular units may contain six hairs in adolescence but only two hairs some years later, when AGA has developed. One treatment option for AGA is the replacement of follicular units by transplantation. These transplants are usually taken from the occiput and thus far the experience of the surgeon decided how much donor skin is taken for each hair transplantation. Ideally, however, a surgeon would calculate the number of follicular units necessary in bald regions and in addition calculate the density of follicular units in donor

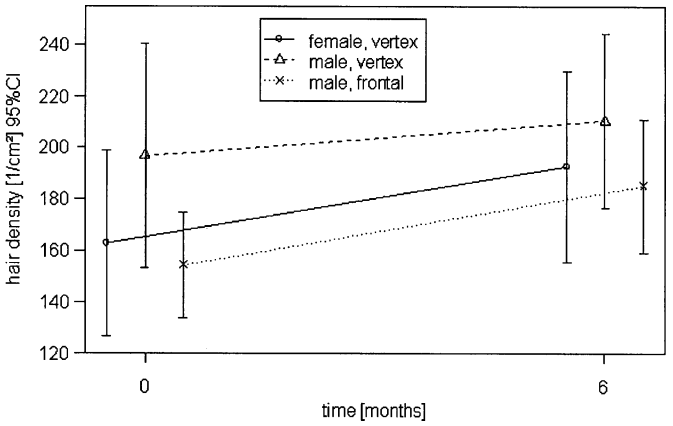

Figure 1
Hair densities after treatment with minoxidil. TrichoScan: hair counts and cumulative hair thickness were analyzed for 6 mo in 10 women and 21 men with AGA treated with 5% minoxidil. In both sexes, at the vertex and in frontal balding in men, an statistically significant increase of hair density has been observed.

areas. In order to analyze the number of follicular units/cm2 we defined the maximal distance of individual hairs, which belong to one follicular unit. With this mathematical approximation we can reveal follicular units, their density and the number of follicular units containing one, two, three or more hairs (Fig 2). In the future this tool may help surgeons to plan a hair transplantation better, in order avoid overtreatment and to preserve as much as donor area as possible.

Human Alopecia Investigation and Research Technology (H.A.I.R. Technology) at Skinterface

Alternative methods have been used at Skinterface (Van Neste *et al*, 1989) that are applicable to a wide range of clinical conditions. In short, after image acquisition (Van Neste *et al*, 1992) and processing by qualified technicians, computer-assisted image analysis generates refined data on disturbed hair growth dynamics in various genetic hair disorders (such as trichrorhinophalangeal syndrome, Sabouraud's triangular alopecia, monilethrix (Van Neste *et al*, 2003) including male and female pattern hair loss. Finally, hair loss increases with aging and hair depigmentation during aging alters hair growth dynamics (Van Neste, 2004; Van Neste and Tobin, 2004).

In a recent paper on female hair loss (Van Neste, in press a), hair growth measurements were collected in 92 female subjects complaining about hair loss. Clinically, they were classified as having a patterned hair loss according to Ludwig (L; n = 50), diffuse hair loss (D; n = 13) or no visible hair loss but complaining of hair shedding (N; n = 29). Two scalp sites on the top of the head and one occipital site were investigated with the contrast enhanced phototrichogram analysis (CE-PTG) (Fig 3).

In female scalp, we confirm that the top of the head shows usually a higher hair density than occipital sites. This physiological observation has also been found in male scalp.

Interestingly, in affected patients (Ludwig pattern (L) and diffuse hair loss (D)) the relative increase of hair counts after contrast enhancement (CE) was much higher (range 22.4%–28.3%) as compared to apparently unaffected females (N; range 8.2%–9.7%). This increase in hair counts was only due in part to the presence of less pigmented thinning hair (thickness less than 40 μm). Such thin hairs were found in statistically significantly higher proportions in younger patients with mildly severe (grade I) patterned alopecia (Ludwig: L).

More recently, along with the identification of the exogen stage of the hair cycle in animal fur (Milner *et al*, 2002), technological developments at Skinterface helped to clear the "scalp scene" from any other loosely attached elements such as exogen hair (application patent: Skinterface—PCT/EP02/06434; June 12, 2002). This most recent refinement was applied in a recent investigation of 88 other female patients (41 with and 47 without patterned hair loss and using exogen-free CE-PTG; unpublished data). Exogen identification added specific information on a largely unsuspected phenomenon that influences significantly hair counts. From these studies, we concluded that the presence or absence of patterning, the clinical severity of the pattern, the increasing age were all associated with significant regression of scalp hair (decreased hair counts, thinner hair and slower growth rates) in female patients.

Finally, based on these data we suggest a three-step mechanism leading to hair loss as well in female as in male subjects (Van Neste *et al*, 2003):

1. a shortening of the growth phase of the hair cycle with maintained thickness (more shedding);
2. an intermittent production of short thin hair, i.e., morphological evidence of miniaturization;
3. a period of very occasional or almost no hair production (dormant follicles or irreversible follicular atrophy).

Therefore, any successful treatment hair loss should stop or reverse the process of hair follicle miniaturization, increase the number of terminal hair follicles while reducing vellus hair counts (Whiting *et al*, 1999) or increasing the percentage of follicles in the growth phase (Van Neste *et al*, 2000) or improving other parameters that influence the productivity of hair such as growth rate or thickness of anagen hairs. Functional follow-up of distinct hair follicle units using

Table I. Six months treatment with 5% minoxidil increases hair density, cumulative hair thickness, and terminal hair counts in both female and male androgenetic alopecia

Gender	Hair density (1 per cm^2)	Cumulative hair thickness (mm)	Terminal hair count (1 per cm^2)	Vellus hair count (1 per cm^2)
♀, vertex	162.7 *vs* 192.6, $p < 0.0001$	4.21 *vs* 5.00, $p = 0.0177$	158.5 *vs* 189.7, $p = 0.0004$	4.27 *vs* 2.92, $p = 0.541$
♂ vertex	196.9 *vs* 210.4, $p = 0.0446$	5.17 *vs* 5.73, $p = 0.0465$	189.5 *vs* 205.9, $p = 0.316$	7.39 *vs* 4.60, $p = 0.266$
♂, frontal	152 *vs* 184, $p = 0.047$	3.60 *vs* 4.48, $p = 0.011$	146 *vs* 172	6.5 *vs* 7.5

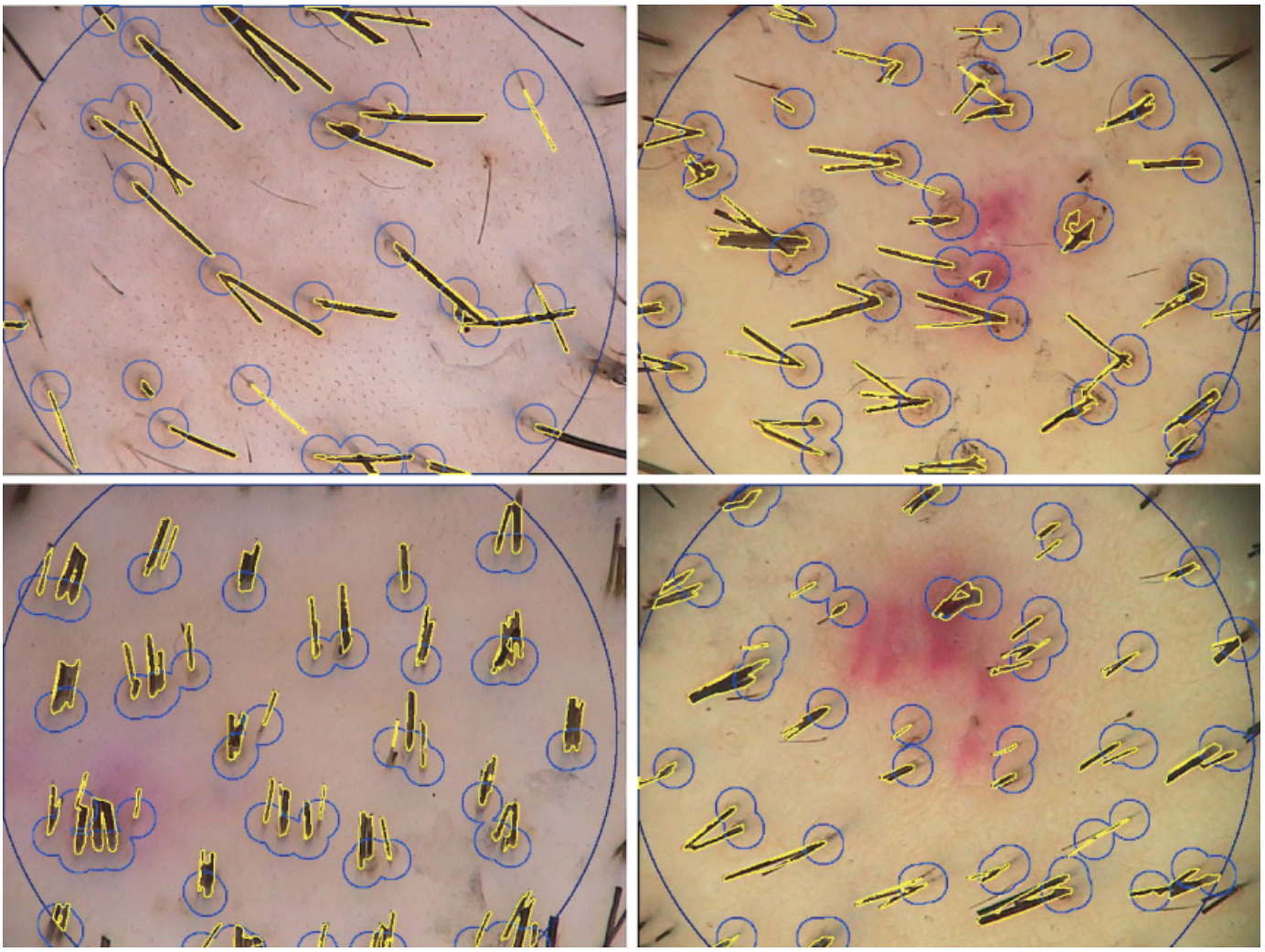

Figure 2
Quantification of scalp hair follicular units with TrichoSan. In order to analyze the number of follicular units per cm^2 we define the maximal distance of individual hairs, which belong to one follicular unit (*blue circle*). With this mathematical approximation we can reveal follicular units, their density and the number of follicular units containing one, two, three, or more hairs. Hairs are marked yellow. A follicular unit is defined with a blue circle. Some units contain only one hair, whereas others have more hairs. In the latter case different blue circles touch each other. Those coalescent blue circles are counted as one follicular unit. This tool may help surgeons to plan a hair transplantation better, in order avoid over treatment and to preserve as much as donor area as possible.

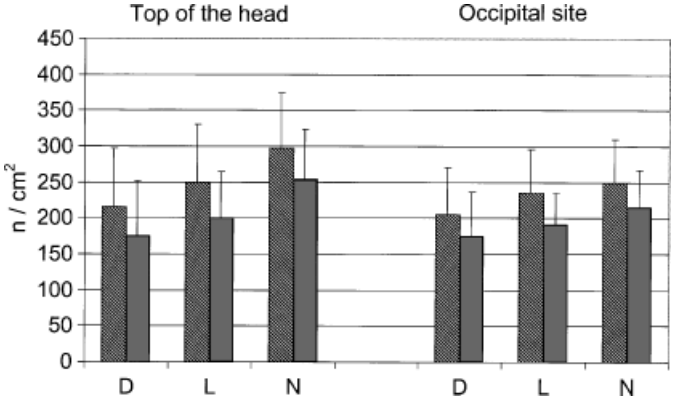

Figure 3
(Adapted from Van Neste in press a) Total and anagen hair density in females complaining of hair loss. Patients were classified clinically as having diffuse hair loss (D), patterned hair loss (Ludwig type; L) or apparently not affected (N). Average values (n per cm^2; with standard deviation) of total (*hatched bars*) and growing (anagen; *gray bars*), i.e., all visible hair using contrast enhanced phototrichogram, was established in the scalp sites under investigation (top of the head (*left panel*) and occipital (*right panel*).

a refined and calibrated method is the only way to clearly document these phenomena (Van Neste, in press, b).

Conclusions

With the TrichoScan an entirely automatic software has been created for the analysis of the aforementioned parameters of hair growth. In rather small clinical trials we show that the TrichoScan is able to reveal the response to treatment with 5% minoxidil in AGA of both sexes. The TrichoScan images are taken either with a video or digital system for ELM. These devices produce high quality and reproducible digital images, because the images are always taken at the same distance of the lens to the skin surfaces. In the future, 5 Megapixel digital cameras might allow to analyze the images even better and with different light sources we will try develop the software, that in the near future a hair dye is no longer necessary. Furthermore we are now able to count the density follicular units, which might be a new tool for hair surgeons (Fig 2).

The refined analytic technology used at Skinterface is along the lines pointed out in previous EHRS meetings on hair measurement techniques (Sinclair *et al*, 2003; Van Neste *et al*, 2003). Recently published results point to the fact that a combination of a highly sensitive and precise analytical approach together with a global calibrated method (Van Neste *et al*, in press) seems advisable in the context of kinetic monitoring of hair growth and hair loss in the hair clinic in general and this is warmly recommended in the context of efficacy analysis of new (and recognized!) compounds in future clinical trials.

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